phy using microbial-derived proteins (e.g. protein A or protein G affinity chromatography), ion exchange chromatography (e.g. cation exchange (carboxymethyl resins), anion exchange (amino ethyl resins) and mixed-mode exchange chromatography), thiophilic adsorption (e.g. with beta-mercaptoethanol and other SH ligands), hydrophobic interaction or aromatic adsorption chromatography (e.g. with phenyl-sepharose, aza-arenophilic resins, or m-aminophenylboronic acid), metal chelate affinity chromatography (e.g. with Ni(II)- and Cu(II)-affinity material), size exclusion chromatography, and preparative electrophoretic methods (such as gel electrophoresis, capillary electrophoresis) (Vijayalakshmi, M. A., Appl. Biochem. Biotech. 75 (1998) 93-102). In one embodiment said chromatography column packing is a chromatography material selected from an affinity chromatography material, or an ion exchange chromatography material, or a thiophilic adsorption chromatography material, or a hydrophobic interaction chromatography material, or an aromatic adsorption chromatography material, or a metal chelate affinity chromatography material, or a size exclusion chromatography material.

In another embodiment the method as reported herein is used for the determination if the process hardware except the chromatography material has reduced separation efficacy.

The following examples and figures are provided to aid the understanding of the present invention, the true scope of which is set forth in the appended claims. As the polypeptide erythropoietin was available in sufficient quantities in our laboratory at the time the invention was made the invention is exemplified with this polypeptide. This should not be understood as limitation but only as an example of the invention. It is understood that modifications can be made in the procedures set forth without departing from the spirit of the invention.

DESCRIPTION OF THE FIGURES

FIG. **1** Experimental data for an exemplary inert change of the conductivity for a re-useable chromatography column packing with no reduced separation efficacy/packing quality (open circles); X-axis: time [min]; Y-axis: conductivity [mS/cm].

FIG. **2** Experimental data for an exemplary inert change of the conductivity for a re-useable chromatography column packing with no reduced separation efficacy/packing quality (open circles) and the fitted function according to a function of formula I; X-axis: time [min]; Y-axis: conductivity [mS/cm].

FIG. **3** Absolute difference between the experimental data for an exemplary inert change of the conductivity for a re-useable chromatography column packing with no reduced separation efficacy/packing quality (open circles) and the fitted function according to formula I; X-axis: time; Y-axis: difference.

FIG. **4** Experimental data for an exemplary inert change of the conductivity for a re-useable chromatography column packing with reduced separation efficacy/packing quality (open circles); X-axis: time [min]; Y-axis: conductivity [mS/cm].

FIG. **5** Experimental data for an exemplary inert change of the conductivity for a re-useable chromatography column packing with reduced separation efficacy/packing quality (open circles) and the fitted function according to a function of formula I; X-axis: time [min]; Y-axis: conductivity [mS/cm].

FIG. **6** Absolute difference between the experimental data for an exemplary inert change of the conductivity for a re-useable chromatography column packing with reduced separation efficacy/packing quality (open circles) and the fitted function according to a function of formula I; X-axis: time [min]; Y-axis: difference [mS/cm].

FIG. **7** Monitoring of column integrity with a method as reported herein over 50 chromatographic cycles using formula II.

FIG. **8** Monitoring of column integrity with a method as reported herein using formula II.

Full circles: parameter derived for regeneration of column without changes in column packing.

Open circles: parameter derived for regeneration of column with a cracked bed during regeneration cycle No. 9.

EXAMPLE 1

Fermentation and Purification of Erythropoietin

Erythropoietin can be produced and purified e.g. according to WO 01/87329.

The purification comprises some chromatography steps. One of these is a Blue Sepharose chromatography. Blue Sepharose consists of Sepharose beads to the surface of which the Cibacron blue dye is covalently bound. Since erythropoietin binds more strongly to Blue Sepharose than most non-proteinaceous contaminants, some proteinaceous impurities and PVA, erythropoietin can be enriched in this step. The elution of the Blue Sepharose column is performed by increasing the salt concentration as well as the pH. The column is filled with Blue Sepharose, regenerated with NaOH and equilibrated with equilibration buffer (sodium/calcium chloride and sodium acetate). The acidified and filtered fermenter supernatant is loaded. After completion of the loading, the column is washed first with a buffer similar to the equilibration buffer containing a higher sodium chloride concentration and consecutively with a TRIS-base buffer. The product is eluted with a TRIS-base buffer and collected in a single fraction in accordance with the master elution profile.

During the equilibration, separation, and regeneration step of the chromatography cycle the conductivity of the mobile phase at the outlet of the column is determined and recorded with a standard conductivity measuring device.

EXAMPLE 2

Change in Column Properties

The column can be monitored over the process continuously using the method as reported herein. Subtle changes become detectable independently of changes of other process parameters. In FIG. **7** a change in the column packing resulting in a change in the separation properties is shown. The change occurred after cycle **40**.

In the case of a column with a broken bed, the quality of the fit decreases dramatically, as seen in the derived parameter residuals difference as shown in cycle **9** of FIG. **8** (open circles).

The invention claimed is:

1. A method for determining whether a re-useable chromatography column packing, which is used at least for the second time in a purification step of a purification of a polypeptide, has reduced separation efficacy in said purification step of said purification of said polypeptide, wherein said method comprises the following steps:

a) identifying and determining the experimental data of an inert change of at least one physicochemical parameter